THE DETERMINATION OF NITRATES IN POLLUTED STREAM AND SEWAGE SAMPLES WITH BRUCINE SULFATE USING THE BRUCINE TECHNIQUE

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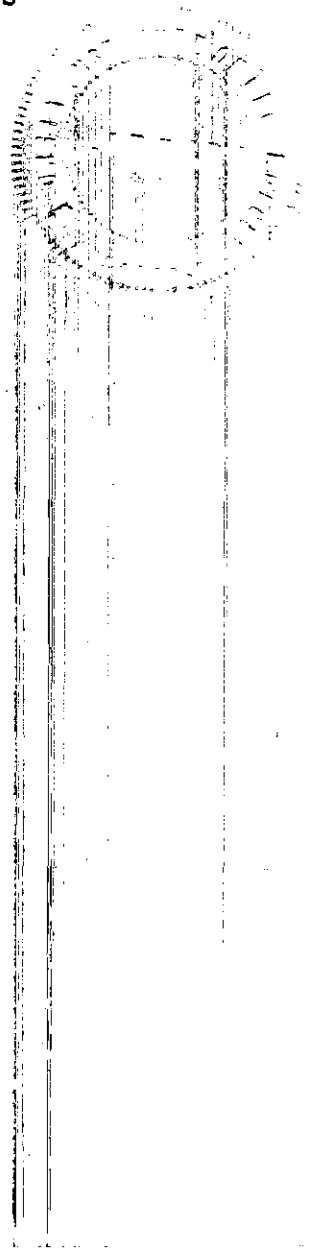

In Partial Fulfillment

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Master of Science in Chemistry

by

William Stewart Johnson

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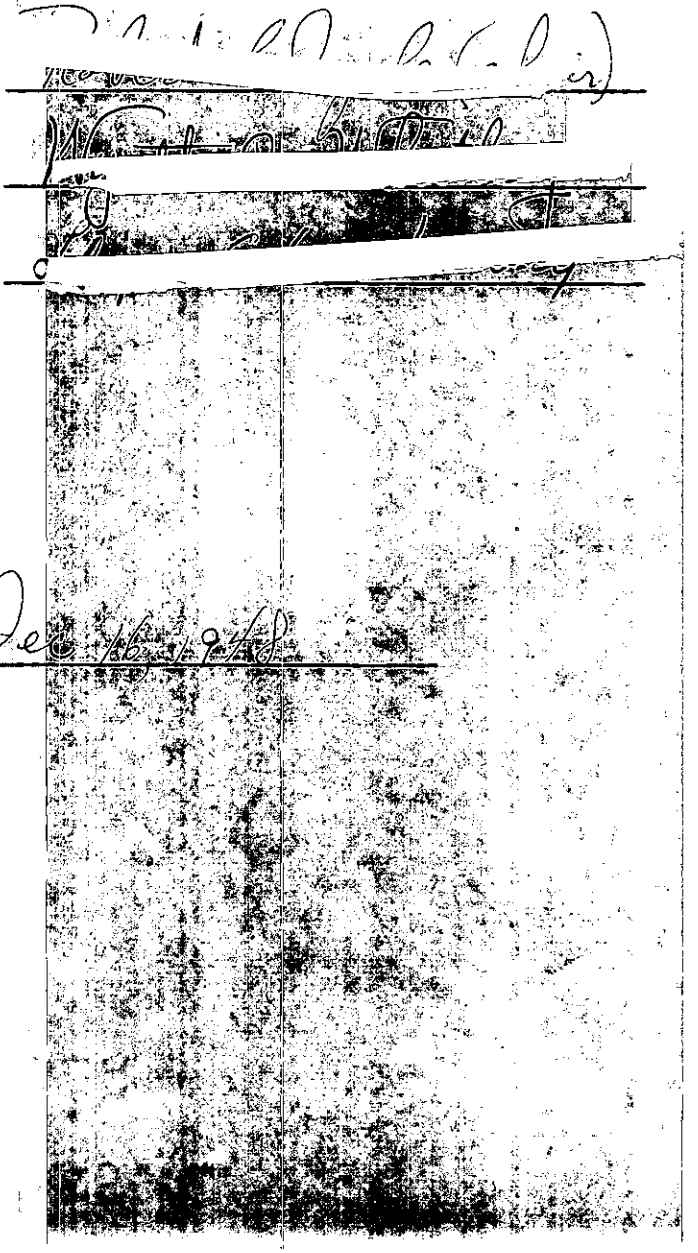

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ACKNOWLEDGMENTS

On completion of this work I wish to express my sincerest thanks to Dr. Robert S. Ingols, Research Associate Professor of Chemistry, not only for his suggestion of the problem, but also for his most valuable aid in its prosecution. His complete cooperation has been the source of constant admiration by the author.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

THE DETERMINATION OF NITRATES IN POLLUTED STREAM AND SEWAGE SAMPLES WITH BRUCINE SULFATE USING THE BRUCINE TECHNIQUE

INTRODUCTION

The approved methods for nitrate determination of water and sewage samples are two in number.[1] The phenoldisulfonic acid test requires a large sample volume, usually 100 ml., and reaction at dryness which involves a time-consuming evaporation. Reduction followed by Nesslerization takes even longer because of the stipulation that a minimum of four hours exposure in the reducing medium is necessary. Normally the volume is the same as in the phenoldisulfonic acid method but evaporation is carried only to about 20 ml. rather than to dryness.

Many other reagents have been suggested,[2] one of the most promising of which is the alkaloid, brucine. The salt, brucine sulfate, is readily available and, being of similar molecular construction, would be expected to react similarly in the presence of nitrates. Its toxicity is lower than the pure alkaloid and is conveniently handled in the form of needles with seven molecules of hydrated water.

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[1] Standard Methods for the Examination of Water and Sewage, Ninth Edition (New York: The American Public Health Association, 1946), 286 pp.

[2] Weil, B. H., P. E. Murray, G. W. Reid, and R. S. Ingols, Bibliography on Water and Sewage Analysis, Special Report Number 28 (Atlanta, Georgia: State Engineering Experiment Station, Georgia Institute of Technology, 1948), 215 pp.

This study, then, is an attempt to employ a brucine sulfate reagent for the determination of nitrates by investigating its reproducibility with various ions normally present in water and organic matter along with its applicability to polluted stream and treated sewage samples. The results obtained will be compared with those of the standard phenoldisulfonic acid method.

REVIEW OF THE LITERATURE

The first attempt to make the brucine test quantitative was made by West-Knights.[3] The reagent was a brucine-oxalic acid solution reacting at dryness with the sample. This gave a permanent red color suitable for comparison with standards prepared similarly. Lunge and Levoff[4] used 0.2 gms. of brucine dissolved in 100 ml. of concentrated sulfuric acid as their reagent in finding the nitrate content of sulfuric acid. After adding 1 ml. to the sample it became necessary to apply heat to establish the yellow color desired for comparison.

Good results were reported by Winkler[5] using 20 ml. of concentrated sulfuric acid and 1 ml. of a 2% solution of brucine sulfate in water in 10 ml. of sample. The determination was made by adding dropwise to 10 ml. of hot distilled water a standard potassium nitrate solution until a matching of yellow tints was obtained. The brucine sulfate solution became cloudy, however, in a few days unless pure brucine alkaloid and 1-2 drops of concentrated sulfuric acid were added.

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[3] West-Knights, J., "A New Method for the Estimation of Nitrates in Potable Waters," Analyst, 6:56-58, April, 1881.

[4] Lunge, G. and A. Levoff, "Detection and Estimation of Minute Quantities of Nitrogen Acids," Journal of the Society of Chemical Industry, 14:67, 68, January 31, 1895.

[5] Winkler, L. W., "Determination of Nitric and Nitrous Acid in Natural Waters," Journal of the Society of Chemical Industry, 20:937, September 30, 1901.

Kostjamin[6] eliminated nitrite interference by boiling the sample with small quantities of sulfuric and phosphoric acids. Winkler[7] showed that by varying the proportion of sulfuric acid present it was possible to test separately for nitrates and nitrites using the same reagents, namely, brucine and sulfuric acid. Nitrites formed a deep red color when equal volumes of water and acid were present. Two volumes of the acid to one of water are necessary with nitrate to produce a color. However, the interference of nitrite received attention later along with a recommendation for the use of sodium azide to eliminate erroneous values due to its presence.[8] Another method was to dilute the color with concentrated sulfuric acid in the case of nitrates and water for nitrites.[9]

Interference from carbohydrates in concentrations of 1000 ppm due to caramelizing when concentrated sulfuric acid was added has also been investigated.[10]

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[6]Kostjamin, N. N., "Quantitative Estimation of Nitric Acid in Water," Journal of the Society of Chemical Industry, 19:933, October 31, 1900.

[7]Winkler, L. W., "Behavior of Nitric and Nitrous Acids with Brucine and Sulfuric Acid," Analyst, 27:162, 163, May, 1902.

[8]Gad, G., "Determination of Nitrate in the Presence of Nitrite in Water," Gas-und Wasserfach, 81:6, 1938.

[9]Snell, F. D., Colorimetric Methods of Analysis, Vol. I (New York: D. Van Nostrand Co., Inc., 1936), 766 pp.

[10]Gad, G., "Detection and Determination of Nitrates in Water in the Presence of Carbohydrates and Chlorides," Kleine Mitteilunger fur die Mitgleider des Vereins fur Wasser-, Boden-, and Lufthygiene, 15:82, 1939.

Recently, Wolf[11] determined nitrate nitrogen concentrations within 0.1 ppm in the range of 0.1 to 2.0 ppm of nitrate nitrogen with brucine in sulfuric acid prepared just prior to using. These results prompted the use of a more stable reagent, brucine in chloroform, for testing boiler water.[12] The accuracy reported was 0.5 ppm nitrate nitrogen and sensitivity of 0.3 ppm nitrate nitrogen. The method was applied to the photoelectric colorimeter at a wave length of 470 millimicrons to give a nearly straight line plot of scale reading versus concentration up to 55 ppm nitrate. None of the ions normally present in boiler waters was found to interfere. High color concentrations were overcome by dilution. This method also has found acceptance in control work using a slide comparator.[13]

In view of these previous investigations, it was decided to undertake this study with the thought of using a stable reagent for water and sewage analysis which would reduce the time and increase the convenience over the approved methods.

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[11]Wolf, B., "Determination of Nitrate, Nitrite, and Ammonia Nitrogen," Industrial and Engineering Chemistry, Analytical Edition, 16:446, 447, July, 1944.

[12]Noll, C. A., "Determination of Nitrate in Boiler Water by Brucine Reagent," Industrial and Engineering Chemistry, Analytical Edition, 17:426-428, July, 1945.

[13]W. A. Taylor and Company, Baltimore, Maryland.

REAGENTS AND PROCEDURE

Reagents.

1. Brucine sulfate solution. Dissolve 2 gms. of reagent grade brucine sulfate $(C_{23}H_{26}O_4N_2)_2 \cdot H_2SO_4 \cdot 7H_2O$ in 80 ml. of a 1:1 solution of 95% ethyl alcohol and distilled water. This is a nearly saturated solution and gentle heating may be applied to aid in dissolving the brucine sulfate crystals. The solution should be stored at room temperature to prevent recrystallization. If this occurs, redissolve by gently heating the solution. Keep tightly stoppered in a glass bottle or flask. Brucine sulfate is poisonous and should be handled with care.

2. Concentrated sulfuric acid. Use C.P. acid of specific gravity 1.84.

3. Standard nitrate stock solution. Dissolve 1.3710 gms. of pure sodium nitrate crystals in one liter of distilled water. One ml. of this solution contains one mg. of nitrate ion.

4. Standard nitrate solutions. Dilute 10 ml. and 100 ml. of stock solution to one liter. These solutions contain 0.01 and 0.1 mg. nitrate ion per ml., respectively.

The Lumetron Photoelectric Colorimeter, Model 450,[14] was employed for all color comparisons. With this instrument 100 ml. low form Nessler tubes with 150 mm. depth markings containing the unknown solution are inserted into the light path.

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[14]Photovolt Corporation, New York, New York.

Procedure.

To a 5 ml. sample in a 125 ml. Erlenmeyer flask add 0.4 ml. of brucine sulfate solution. Add 10 ml. of concentrated sulfuric acid. To avoid spattering this should be added down the side of the flask slowly while rotating the flask. Swirl to mix. Allow to stand five minutes. Add approximately 60 ml. of distilled water. Cool. Pour contents of the flask into a Nessler tube. The volume is approximately 75 ml. at this point. Rinse the flask several times with small portions of distilled water, adding each portion to the Nessler tube until the sample is diluted to 100 ml. Invert three times to mix. Decrease the volume to 150 mm. depth mark. Insert into colorimeter and record scale reading using a filter with maximum transmittancy at a wave length of 420 millimicrons. Read the nitrate equivalent to the scale reading from the standard curve.

A standard curve was prepared by treating known amounts of standard nitrate solution in a similar manner. Where the volume of standard solution is less than 5 ml. add sufficient distilled water to bring it to this volume. Plot the scale reading against concentration. A sample curve is shown in Figure 1. The insert in the lower right hand corner is an enlargement of the area between 0-2 ppm which will be found helpful in determining very low concentrations.

For best results a solution of known nitrate concentration should be used to "zero" the colorimeter, using the value for this concentration as obtained from the standard curve to check the instrument after each reading.

If the sample contains color, as in the case of sewage, filter through activated charcoal before starting the procedure.

RESULTS

Initial determinations using brucine sulfate and known concentrations of nitrate gave a wide range of results both as to reproducibility and accuracy. The procedure as outlined above has been found to reduce these errors to a point where they are comparable to the standard phenoldisulfonic acid method.

Brucine sulfate crystals dissolved more easily when 1-2 ml. of concentrated sulfuric acid was added to the alcohol-water mixture, but in a few days the solution developed a yellow color and its use was discontinued in favor of the colorless one.

The ratio of two volumes of sulfuric acid to one of water was maintained to prevent nitrite interference. A red color may develop first in the reaction but this is unstable and will give way to the desired yellow color by swirling the solution. Although Table I shows no change results in color intensity between five and twenty minutes elapsed time after addition of acid and dilution the shorter interval was observed in all determinations. Preliminary dilution to approximately 60 ml. preserves the color developed while cooling occurs. Readings should be made within thirty minutes after addition of the acid (Table II).

The choice of a filter whose maximum transmittancy was 420 millimicrons was made from the observations plotted in Figure 2. This curve shows a greater absorption of light at this wave length than at the other wave lengths studied. By the use of color developed in a known solution and its position on the standard curve as the check-setting for the photoelectric colorimeter, the brucine sulfate reagent

was found to be stable for twenty days (Table III). Investigation beyond this period was not made. Brucine sulfate from two different manufacturers[15] gave concordant results.

The effect of interference from ions normally present in streams and sewage was studied from an artificial sample. Table IV gives the analysis of this sample and the results obtained from nitrate determinations by brucine sulfate and phenoldisulfonic acid techniques. Oxygen consumed values, which measure the amount of organic matter, were found for two sewage samples. These results are shown in Table V. In the presence of carbohydrates to the extent of 1000 ppm, the characteristic yellow color may take on an orange tinge when the concentrated sulfuric acid is added. It does not interfere with the final result, however, because upon dilution to 100 ml. the original color is restored. Table VI shows the agreement reached in a 1000 ppm glucose sample.

A stream and several well water samples from the Atlanta area were analyzed for their nitrate content. The results are tabulated in Table VII. Each letter under the column "Sample Designation" represents a single sample and the values to the right are those obtained in simultaneous determinations.

While this investigation was in progress a Biological Oxygen Demand (B.O.D.) study by the dilution technique was made at the Georgia Institute of Technology which provided numerous samples of nitrate

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[15] Eastman Kodak Company, Organic Chemical Division, Rochester, New York. Merck and Company, Incorporated, Rahway, New Jersey.

concentrations lower than those previously mentioned and approaching the concentrations normally found in stream waters.

Fifty-six B.O.D. samples were analyzed for nitrate content both by brucine sulfate and standard phenoldisulfonic acid methods. Ten samples, all having less than .35 ppm nitrate according to brucine sulfate results, were run in duplicate with nine being reproducible within ± 0.1 ppm nitrate and the tenth ± 0.2 ppm nitrate or a reproducibility of about 20% at these low concentrations. This represents, in the former case, ± 0.02 ppm nitrate nitrogen and, in the latter instance, ± 0.04 ppm nitrate nitrogen.

However, comparison of the standard method results of the fifty-six determinations showed sharp disagreement with those obtained by brucine sulfate. Only twenty-three were within ± 0.1 ppm nitrate; eight within ± 0.2 ppm nitrate; six within ± 0.3 ppm nitrate; four within ± 0.4 ppm nitrate; and the remaining fifteen ± 0.5 ppm nitrate or more. This lack of agreement was apparent from the beginning of the B.O.D. study and was remedied only by using a 420 millimicron filter rather than 490 millimicrons for the phenoldisulfonic acid results. Justification for this substitution has been obtained from an investigation of the spectrophotometric character of nitrosubstituted phenoldisulfonic acid under various concentrations of color developed. (This is an important problem but it is outside the scope of this thesis and will therefore be omitted.) For the final twenty-eight nitrate determinations of B.O.D. samples, values were obtained at both wave lengths. Those at 490 millimicrons have already been discussed.

When these twenty-eight results obtained at 420 millimicrons were compared with simultaneous brucine sulfate determinations of nitrate content, eighteen showed a difference of less than ± 0.1 ppm nitrate; six showed ± 0.1 ppm nitrate difference; two showed ± 0.2 ppm nitrate difference; one showed a - 0.3 ppm nitrate variation from brucine sulfate result; and one a + 0.4 ppm nitrate variation from brucine sulfate result. The actual nitrate concentrations in all of these samples was less than 0.5 ppm nitrate, so that the percentage error is approximately in the range of reproducibility at low concentrations.

An investigation was made by concentrating eleven of the samples to test the increased accuracy of a higher concentration. One hundred milliliters of B.O.D. sample were evaporated to dryness and the residue redissolved in 10 ml. of distilled water. This increased the nitrate concentration ten times. Five milliliters of this was then used to make a brucine sulfate determination of its nitrate content. Of the eleven samples treated in this manner, ten were within -0.04 ppm nitrate of the previously determined standard method value in the original sample (Table VIII). However, in general the brucine sulfate values are lower than the phenoldisulfonic acid values even though in good agreement.

DISCUSSION

The method does not apply directly to solutions of nitrate concentrations greater than 10 ppm. Samples must be diluted to bring the concentration into the range of 0-10 ppm. The section of the standard curve above 10 ppm could not be reproduced and the onset of its deviation from a straight line occurred throughout the range of 12-16 ppm. Noll,[16] employing brucine in chloroform, used a filter of maximum transmittance of 470 millimicrons to overcome this limitation. With brucine sulfate reagent the difference in scale readings at 470 millimicrons was too small to consider its use. Since streams and sewage normally are low in nitrates it is more advantageous to have a wide scale variation in this range of concentration rather than a wide range of concentration and narrow scale variation.

The stability of the color produced cannot begin to compare with that of the standard technique. Similarly, reagents used in the phenoldisulfonic acid method are not subject to any appreciable deteriorations with time, while with brucine sulfate it is necessary to "zero" the colorimeter with a known standard color to give stability over the period of time studied and to renew the brucine sulfate reagent at regular intervals.

Organic matter including carbohydrates will not effect reproducibility and accuracy up to 350 ppm of heterogeneous organic matter present in sewage and 1000 ppm glucose. None of the ions investigated interfere

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[16] Noll, C. A., Loc. Cit.

in the concentrations in which they are found normally in stream and sewage samples.

The high percentages of error in reproducibility and accuracy encountered in samples low in nitrate content were caused in part by the limitations of the photoelectric colorimeter. In the area of the scale where these readings occur the instrument does not exhibit its greatest sensitivity. Manufacturers' instructions which accompany the colorimeter cover these limitations. From the samples analyzed, it appears that the best range of concentration for the brucine sulfate procedure is about 0.7-2.0 ppm. Further, with the exception of low concentrations, reproducibility and accuracy may be considered to be approximately the same as the standard phenoldisulfonic acid method.

The requirement of reaction at dryness for phenoldisulfonic acid and, therefore, a time consuming evaporation, has been eliminated with brucine sulfate reagent. Ten determinations can be completed in less than one hour with brucine sulfate. Sample size has been reduced from 100 ml. to 5 ml.

SUMMARY AND CONCLUSIONS

A colorimetric procedure for the determination of nitrates in stream and treated sewage samples has been presented. Color is produced by adding a solution of brucine sulfate in ethyl alcohol and water to the sample followed by the addition of concentrated sulfuric acid. The reagent and the color developed are not as stable as in the standard phenoldisulfonic acid method. In concentrations above 10 ppm, dilution before color development is necessary. Results are comparable to those of the phenoldisulfonic acid technique, particularly between about 0.7-2.0 ppm nitrate. Lower concentrations are subject to deviations because of the small amount of color along with the limitations of the photoelectric colorimeter. Organic matter including carbohydrates and various ions do not interfere within the limits investigated. Procedure time and sample size have been decreased over the present approved techniques. The procedure with brucine sulfate is therefore recommended for consideration as a standard method for nitrate determination.

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APPENDIX I

TABLES

TABLE I

EFFECT OF TIME AFTER ADDITION OF ACID AND BEFORE DILUTION

Nitrate Present (ppm)	Time After Addition of Acid (min)	Nitrate Found (ppm)
10.0	0	20.0
10.0	5	10.0
10.0	10	10.0
10.0	20	10.0

TABLE II

STABILITY OF COLOR DEVELOPED

Nitrate Present (ppm)	Time After Addition of Acid (min)	Nitrate Found (ppm)
10.0	15	10.0
10.0	30	9.8
10.0	45	9.3
10.0	60	8.5
10.0	120	6.5

TABLE III

STABILITY OF REAGENT

Nitrate Present (ppm)	Age of Reagent (Days)	Nitrate Found (ppm)
2.0	$\frac{1}{2}$	2.0
1.2	3	1.2
0.2	7	0.2
10.0	7	10.0
6.0	14	6.0
2.0	20	2.1
10.0	20	10.0
10.0	20	9.8

TABLE IV

EFFECT OF IONS NORMALLY PRESENT IN STREAM AND SEWAGE SAMPLES

Ions and Their Concentrations (ppm)		
Ca^{++} = 50	NO_3^- = 10	Fe^{++} = 1
Mg^{++} = 25	NH_4^+ = .6	HCO_3^- = 100
Cl^- = 90	NO_2^- = .5	
PO_4^{---} = .05	SO_4^{--} = 100	

Nitrate found by Brucine Sulfate Technique (ppm)	Nitrate found by Phenoldisulfonic Acid Technique (ppm)
10.2	10.0
10.3	9.9
10.2	
10.2	

TABLE V

COLOR DEVELOPMENT IN THE PRESENCE OF ORGANIC MATTER

Nitrate found by Brucine Sulfate Technique (ppm)	Nitrate found by Phenoldisulfonic Acid Technique (ppm)	Oxygen Consumed(ppm)
.75	——	350
.70	——	350
1.4	1.1	326
1.2	1.1	326

TABLE VI

COLOR DEVELOPMENT IN THE PRESENCE OF CARBOHYDRATES

Nitrate Present (ppm)	Glucose Present (ppm)	Nitrate Found (ppm)
10.0	1000	9.8
10.0	1000	9.9

TABLE VII

NITRATE DETERMINATIONS OF WELL WATERS AND STREAM SAMPLE

Sample Designation	Nitrate found by Brucine Sulfate Technique (ppm)	Nitrate found by Phenoldisulfonic Acid Technique (ppm)
A	14	14
	14	13
B	20	12
	18	12
C	1.7	1.3
	1.6	1.7
	1.5	
	1.3	
D	6.7	6.4
	6.5	
	6.8	
E	2.7	2.7
	2.8	2.8
F	1.5	1.4
	1.4	1.1
G[17]	1.7	1.9
	2.0	
Stream Sample	2.0	1.5
	2.0	2.0

[17] To 90 ml. of a well water which showed 1.3 ppm nitrate by brucine sulfate technique were added 5 ml. of distilled water and 5 ml. of a solution which was known to contain .01 mg. NO_3 per ml. The resulting solution would then have a concentration of 1.7 ppm nitrate. The values given are for this solution.

TABLE VIII

COMPARISON OF NITRATE DETERMINATIONS OF CONCENTRATED B.O.D. SAMPLES

Original Nitrate Content As Determined from Concentrated Sample by Brucine Sulfate Technique (ppm)	Original Nitrate Content As Determined by Phenoldisulfonic Acid Technique of 420 Millimicrons (ppm)[18]	Difference (ppm)
0.14	0.17	-0.03
0.08	0.11	-0.03
0.07	0.11	-0.04
0.10	0.13	-0.03
0.16	0.17	-0.01
0.20	0.19	+0.01
0.15	0.16	-0.01
0.19	0.52	-0.33
0.10	0.12	-0.02
0.10	0.12	-0.02
0.03	0.06	-0.03

[18] Phenoldisulfonic acid results at 490 millimicrons showed same disagreement as noted in the text.

APPENDIX II

FIGURES

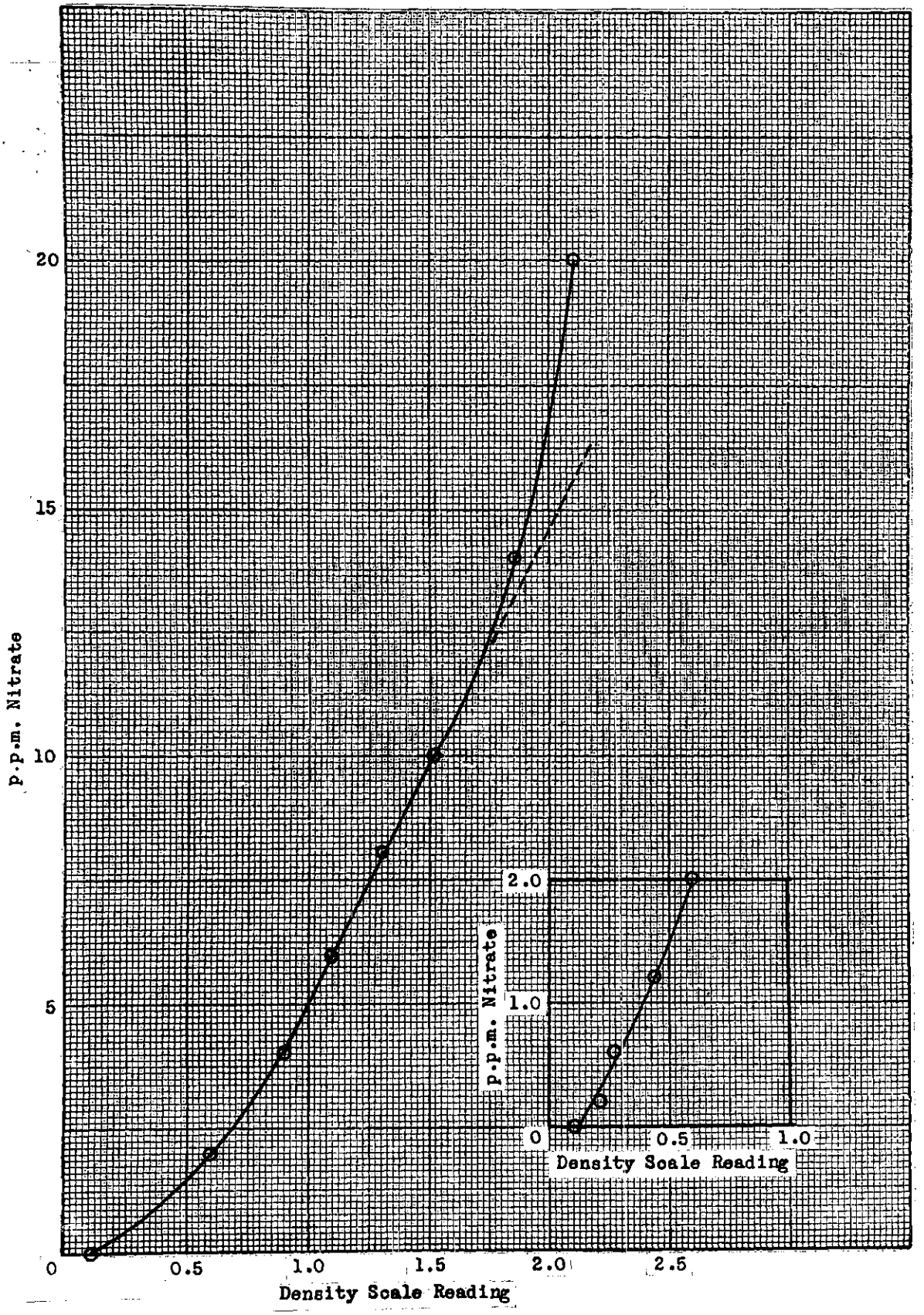

FIGURE 1. SAMPLE STANDARD CURVE FOR PHOTOELECTRIC COLORIMETER

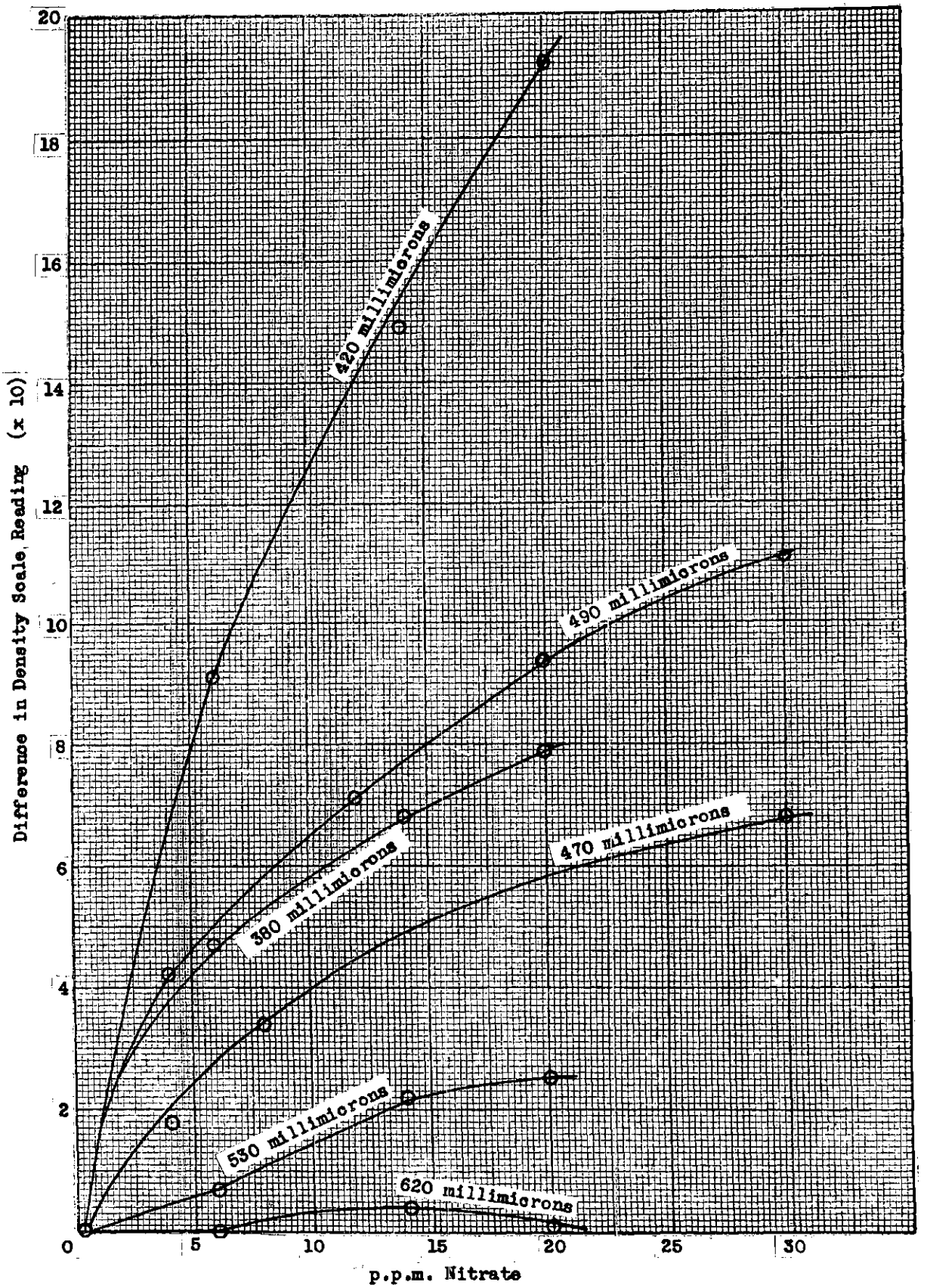

FIGURE 2. GRAPH SHOWING RANGE OF DENSITY SCALE READINGS WITH FILTERS HAVING MAXIMUM TRANSMITTANCY AT VARIOUS WAVE LENGTHS.